



Original communication

Lethal hypothermia in an animal model, not associated with basal renal epithelial vacuolization



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ABSTRACT

A rodent model was used to evaluate the association between hypothermia and basal vacuolization in renal tubular epithelial cells. 28 Sprague Dawley rats were anaesthetized in non-stressful conditions and placed two at a time into a cooling chamber. Body core temperatures dropped to a minimum of 7–10 °C, causing death under anaesthesia at times varying from 120 to 240 min. The animals were then subjected to necropsy; the kidneys were removed and placed in 10% buffered formalin. Examination of haematoxylin and eosin-stained renal sections failed to reveal basal vacuolization of renal tubular epithelial cells in any of the 28 animals. In this model, no evidence of subnuclear lipid vacuolization of renal tubular cells could be demonstrated despite significant and eventually lethal hypothermia. These results lend support to the hypothesis that the basal vacuolization in hypothermia may be a manifestation of a more complex pathophysiological pathway rather than being due simply to low body core temperatures.

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1. Introduction

Deaths from hypothermia are encountered in forensic mortuaries in both temperate and cold climates.¹ Unfortunately the findings at autopsy, although characteristic, are not diagnostic, and their underlying pathophysiology is poorly understood.² Typical features include Wischnewsky spots of the gastric mucosa in 40–90% of cases, pinkish discolouration of the skin over larger joints, and acute pancreatitis with haemorrhage and surrounding fat necrosis.^{3,4} Microscopic findings have included vacuolization of hepatocytes, cardiac myocytes and renal tubular epithelial cells.^{4,5} It has been suggested that basal lipid vacuolization in renal epithelial cells could serve as a potential diagnostic tool for identifying hypothermic deaths, as it has an “equal value of diagnostic sensitivity compared to that of Wischnewski-ulcers”.⁵

However, basal vacuolization of renal tubular epithelial cells may be also caused by underlying metabolic derangements, rather than by the direct effects of hypothermia.^{6–9} A recent study by the authors demonstrating basal vacuolization in 15 out of 46 cases of

hypothermia (33%), revealed that 9 of the 15 cases (60%) had a documented history of diabetes mellitus (which is known to be associated with basal vacuolization).⁶ Vitreous humour biochemical analyses were performed in seven of these cases, all of which demonstrated diabetic ketoacidosis.⁶ Based on these findings we suggested that diabetic ketoacidosis may be a more significant factor in the generation of basal vacuolization than hypothermia in isolation.⁶ We also noted that hypothermia might, in fact, be both a cause and a complication of diabetic ketoacidosis, which accounts for 11.8% of hospital admissions of all patients with markedly low core temperatures.^{6,10} The same may apply to alcoholic ketoacidosis, which is also known to be associated with the formation of renal basal vacuolizations^{13,14}; e.g. a study of 51 fatal hypothermic deaths in Sweden revealed that in 47% of cases the subjects were long-term alcoholics, and that 65% had detectable levels of ethanol in the blood and/or urine.¹¹

We chose to use a rat model to specifically test whether basal vacuolization of renal tubular epithelial cells could be caused by lethal hypothermia in isolation.

2. Materials and methods

A model using male Sprague Dawley rats was developed to assess the effects of hypothermia in anaesthetized, minimally-

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stressed animals.¹² Twenty-eight rats were given an acclimatization period of one week with free access to food, water, and social interaction to reduce psychological and physiological stress. They were then sequentially anaesthetized with isoflurane at a flow rate of 3–5% and an oxygen flow rate of 1–2 ml/min prior to placement into a cooling chamber. The isoflurane flow rate was decreased to 1–2% during the procedure to avoid significant respiratory depression whilst maintaining deep sleep. Constant monitoring of body core temperatures was achieved using a rectal temperature probe until death occurred. Core temperatures dropped over time to a minimum of 7–10 °C which resulted in death of the animals under anaesthesia at times ranging from 120 to 240 min after placement in the cooling chamber. The animals were then subjected to necropsy and the kidneys removed and immediately placed in 10% buffered formalin. Routine haematoxylin and eosin-stained slides were then prepared and examined. This study was fully approved by the Animal Ethics Committee at The University of Adelaide.

3. Results

Microscopic examination of coronal sections through each kidney from all 28 animals failed to reveal any evidence of basal vacuolization in the renal tubular epithelial cells. Although occasional sections displayed areas of non-specific, irregular cytoplasmic vacuolization, sections of the kidneys were generally unremarkable. (The latter vacuolization was thought to represent an artefact due to delayed fixation of deeper tissues.)

4. Discussion

Although lipid-containing vacuoles have been found in the basal portions of renal tubular epithelial cells in cases of hypothermia in humans^{5,6} the pathophysiology remains uncertain. It appears likely that such deposits may occur when there has been a significant metabolic disruption resulting in lipid mobilization from tissue stores, as occurs with ketoacidosis arising from a number of different conditions including diabetes mellitus, alcoholism and starvation.^{13–16} Over the last decade, the term “Armanni-Ebstein phenomenon” has been applied broadly to include descriptions of subnuclear lipid vacuolizations of renal tubular epithelial cells in the outer medulla; however, a recent review has clarified the precise use of the Armanni-Ebstein term, indicating that this classification should refer specifically to clear cell changes of swollen, rounded and transparent cells having peripherally displaced nuclei associated with hyperglycemia.¹⁷

In the present study there was no evidence of Armanni-Ebstein changes, nor of subnuclear lipid vacuolization of tubular cells, despite the imposition of significant and eventually lethal hypothermia. The rats used in this study had been subject to minimal psychological and physiological stress, and had been in good physical condition, therefore creating a suitable model to study the effect of hypothermia in isolation.¹²

A possible limitation of this study is that the rat model might not develop the subnuclear vacuolization phenotype. However, studies have shown that rats exposed to hypothermic conditions under stress do demonstrate other features associated with hypothermia such as Wischnewsky spots of the gastric mucosa.^{18–22} Sprague Dawley rats have also been successfully used in previous studies to manifest other renal histological changes such as Armanni-Ebstein lesions.^{23–26} These studies suggest the rat is a valid animal model for exploring renal sequelae of pathophysiological conditions. A second possible limitation is that the time of cold exposure may have been too short to allow for the development of subnuclear

vacuolization; however some animals did survive for hours with extremely low core temperatures. Of note, the rats in this study also did not develop Wischnewsky spots, which could be directly related to the role that stress may have in the pathological manifestations of hypothermia.¹²

This study suggests that basal renal tubular cell vacuolization may not be caused solely by lethal hypothermia. In cases where renal pathology is observed following significant hypothermia, additional studies testing for underlying metabolic disturbances, perhaps involving lipid mobilization due to ketoacidosis, will be required to determine the potential role of disturbances of glucose metabolism associated with lowered core temperatures, in creating this phenotype.

Ethical approval

The University of Adelaide Animal Ethics Committee.

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Conflict of interest

None.

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